Pathophysiological aspects of lifestyle and the kidney

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CHAPTER 10

Vascular endothelial growth factor C levels are modulated by dietary salt intake in proteinuric chronic kidney disease and healthy subjects

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ABSTRACT

Introduction. Recent experimental findings demonstrate vascular endothelial growth factor C (VEGF-C) mediated water-free storage of salt in the interstitium, which prevents a salt-sensitive blood pressure state. It is unknown whether this mechanism plays a role in salt homeostasis and regulation of blood pressure in humans as well. Therefore, we investigated circulating VEGF-C levels and blood pressure during different well-controlled salt intakes in healthy subjects and in chronic kidney disease (CKD) patients.

Material and Methods. In two cross-over studies, healthy subjects (n=31) and non-diabetic proteinuric CKD patients (n=32) were treated with consecutively a low sodium diet (LS, aim 50 mmol Na^+/d) and a high sodium diet (HS, aim 200 mmol Na^+/d) in random order, during two 1-week (healthy subjects) and two 6-week periods (CKD patients).

Results. We found that VEGF-C levels are higher during HS than during LS in CKD patients (P=0.034) with a trend towards higher VEGF-C in healthy subjects as well (P=0.070). In CKD patients, HS was associated with higher NT-proBNP levels (P=0.005) and body weight (P=0.013), consistent with ECV expansion, and with higher blood pressure (P<0.001), indicating salt-sensitivity. In healthy subjects, blood pressure was not affected by dietary salt (p=0.14), despite a rise in ECV (P=0.023).

Conclusion. Our findings support a role for VEGF-C mediated salt homeostasis in humans. Considering the salt-sensitivity of blood pressure, this buffering mechanism appears to be insufficient in proteinuric CKD patients. Future studies are needed to provide causality, and to substantiate the clinical and therapeutic relevance of this VEGF-C regulatory mechanism in humans.

INTRODUCTION

Classically, total body salt and extracellular volume (ECV) are thought to be closely linked and controlled by renal salt excretion and dietary salt intake only. Based on the assumption that extracellular body fluids are in equilibrium, excess interstitial salt is considered to be readily mobilized into the bloodstream for renal salt clearance. Blunted renal salt excretion in this concept results in ECV expansion, which can induce a rise in blood pressure, denoted as the salt-sensitivity of blood pressure.[1,2] In support of this concept we found that salt-sensitive healthy men have a higher ECV than salt-resistant men during high salt intake, but not during low salt intake.[3]

However, recent experimental findings demonstrating water-free storage of salt, question our current understanding on internal environment composition and warrant novel insights into regulatory mechanisms for salt homeostasis.[4-9] Salt can be stored in a newly discovered subcutaneous interstitial compartment, by binding to polyanionic proteoglycans and glycosaminoglycans without commensurate water retention.[10,11] In response to salt-mediated interstitial osmotic stress, mononuclear phagocyte system cells secrete vascular endothelial growth factor C (VEGF-C), which stimulates lymphatic growth and endothelial nitric oxide synthase (eNOS) expression.[12,13] When this system is inhibited, high salt intake induces excess interstitial salt retention and hypertension.[4,5]

In patients with refractory hypertension, a condition which is eminently salt-sensitive,[14,15] circulating VEGF-C levels were elevated compared to normotensive subjects,[4] suggesting that this extrarenal regulatory mechanism might play a role in salt homeostasis and regulation of blood pressure in humans as well. If so, it can be hypothesized that circulating levels of VEGF-C respond to changes in salt intake, with higher VEGF-C levels during high salt intake. To test this hypothesis, we investigated circulating VEGF-C levels and blood pressure during steady state on different well-controlled salt intakes in two independent studies, in proteinuric chronic kidney disease (CKD) patients and in healthy volunteers, respectively.

MATERIALS AND METHODS

This is a post-hoc analysis of two previous studies described in detail elsewhere.[3,24]

CKD patients

For the current study, we used data and samples collected during placebo-treatment on high sodium (HS; target intake 200 mmol Na^+/d) and low sodium diet (LS; target intake 50 mmol Na^+/d) from 32 non-diabetic proteinuric CKD patients (age 50±2 years, 73% men, all Caucasian, BMI 27±1 kg/m^2). Mean achieved sodium intake was above target (90±10 mmol/d) and according to protocol (200±10 mmol/d) during LS and HS diet, respectively. Duration of the dietary interventions was two times 6 weeks, and the order was random. For

2 subjects from the original study, good quality samples were no longer available.

Healthy subjects

From the original 34 study subjects, samples of sufficient quality were available for 31 subjects (age 23±1, 100% men, all Caucasian, BMI 24±1 kg/m^2). Data and samples were obtained after one week on a low sodium diet (LS; target intake 50 mmol Na^+/d) and after one week on a high sodium diet (HS; target intake 200 mmol Na^+/d), respectively, in random order. Mean achieved dietary sodium intake was below and above target values (34±11 mmol/d and 257±16 mmol/d, respectively) during LS and HS diet, respectively.

Measurements and calculations

At the end of each study period all participants collected 24 hour urine and, after an overnight fast, blood pressure was measured and blood was sampled. Proteinuria was measured by the pyrogallol red-molybdate method. Dietary sodium intake was assessed from 24 hour urinary sodium excretion. Blood pressure was measured at 1-minute intervals by an automatic device (Dinamap®; GE Medical Systems, Milwaukee, WI), with the patient in semi-supine position. After fifteen minutes of measurements, the mean of the last four readings was used for further analysis. Plasma VEGF-C levels were measured by ELISA (R&D Systems, Germany). Intra- and interassay variation of the ELISA is 6.6% and 8.5%, respectively. The minimal detection level is 48.4 pg/mL. In the healthy subjects ECV was measured by the distribution volume of ^{125}I-iothalamate as described previously.[25]

Data analysis

Data are given as mean with SEM, or geometric mean with interquartile range (IQR) when skewed. Before statistical testing, skewed variables were natural log-transformed to obtain normality. Comparisons between HS and LS were performed using paired T-tests. $P<0.05$ was considered statistically significant. SPSS 18.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for all analyses.

RESULTS

CKD patients had overt proteinuria, a slightly elevated blood pressure, and a rather preserved renal function (Table 1). Urinary sodium excretion, a measure of dietary sodium intake, was lower during LS than during HS. VEGF-C levels were significantly higher during HS than during LS (median (IQR) 1228 (1024-1471) vs. 1004 (857-1177) pg/mL, respectively, p=0.034; Figure 1). NT-proBNP levels and body weight were also higher during HS than during LS, consistent with ECV expansion during HS. Blood pressure and proteinuria were higher during HS as well, indicating salt-sensitivity of blood pressure and proteinuria in CKD patients.

Table 1. General parameters in CKD patients (N=32) during low and high sodium diet.

	LS	HS	P
Proteinuria (g/d)	3.0±0.4	3.8±0.4	<0.001
Systolic blood pressure (mmHg)	137±3	143±3	<0.001
Diastolic blood pressure (mmHg)	83±1	86±2	0.004
Mean arterial pressure (mmHg)	101±11	105±15	0.001
Creatinine clearance (mL/min)	82±6	89±5	0.217
NT-proBNP (pg/mL)	62 (41-93)	91 (60-137)	0.005
Body weight (kg)	89.1±2.9	91.2±3.0	0.013
Plasma VEGF-C (pg/mL)	1004 (857-1177)	1228 (1024-1471)	0.034
Plasma Na^+ (mmol/L)	139.0±0.4	139.1±0.4	0.666
Urinary Na^+ excretion (mmol/d)	90±10	200±10	<0.001

Abbreviations: LS: low sodium diet, HS: high sodium diet.

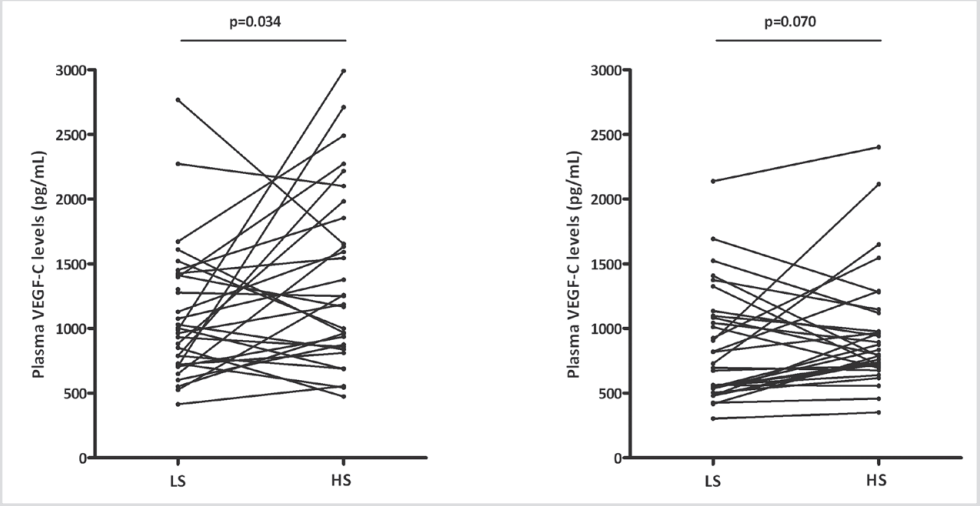
As expected, the healthy subjects had normal blood pressure, normal renal function and no proteinuria (Table 2). Urinary sodium excretion was considerably lower during LS than during HS, indicating excellent dietary compliance. VEGF-C levels tended to be higher during HS than during LS, but the difference was not statistically significant (median (IQR) 881 (758-1023) vs. 773 (748-921) pg/mL, respectively, p=0.070; Figure 1). Assuming that VEGF-C distributes over the ECV, we calculated the total amount of VEGF-C. as product of plasma VEGF-C levels X ECV. Total VEGF-C was higher during HS than during LS (median (IQR) 18176 (14320-26405) vs. 14539 (1002-22751) pg, respectively, p=0.016). In line with the higher ECV during HS, NT-proBNP levels, body weight, and creatinine clearance were also significantly higher during HS than during LS. Blood pressure in the healthy young men was not affected by dietary salt intake.

Table 2. General parameters in healthy subjects (N=31) during low and high sodium diet.

	LS	HS	P
Proteinuria (g/d)	<0.2	<0.2	-
Systolic blood pressure (mmHg)	123±2	124±1	0.138
Diastolic blood pressure (mmHg)	68±1	69±1	0.453
Mean arterial pressue (mmHg)	86±8	87±7	0.251
Creatinine clearance (mL/min)	103±5	123±5	0.003
NT-proBNP (pg/mL)	14 (11-19)	26 (20-35)	0.002
Body weight (kg)	79.9±2.1	81.6±2.1	<0.001
Extracellular volume (L)	19.8±0.5	20.8±0.5	0.023
Plasma VEGF-C (pg/mL)	773 (748-921)	881 (758-1023)	0.070
Total amount of VEGF-C (pg)	14539 (1002-22751)	18176 (14320-26405)	0.016
Plasma Na+ (mmol/L)	138.5±0.4	139.8±0.4	0.001
Urinary Na+ excretion (mmol/d)	46±11	257±16	<0.001

Abbreviations: LS: low sodium diet, HS: high sodium diet.

Figure 1. VEGF-C levels in CKD patients (N=32, left panel) and healthy subjects (N=31, right panel) during low and high sodium diet.



Reference value NT-proBNP <125 ng/L. Abbreviations: LS: low sodium diet, HS: high sodium diet, VEGF-C: vascular endothelial growth factor C.

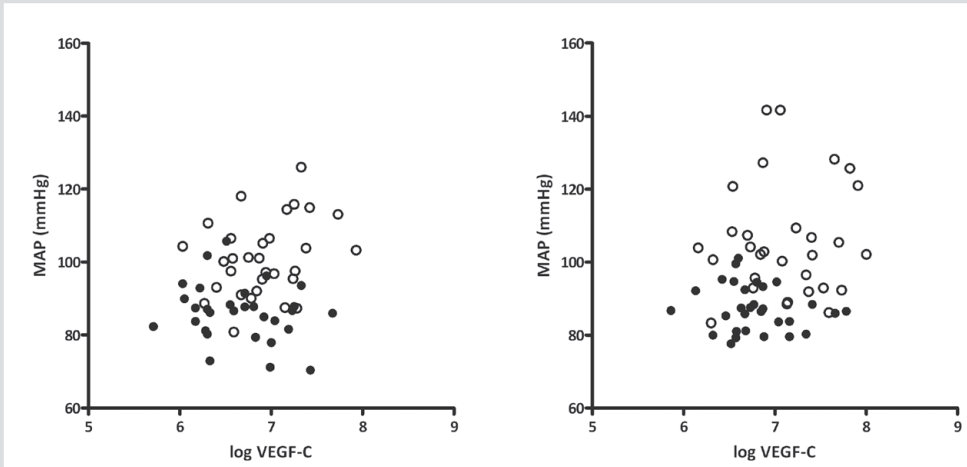
Individual values for blood pressure and VEGF-C during LS and HS in the CKD patients and the healthy subjects are given in Figure 2. No significant correlation could be detected in the healthy subjects nor in the CKD patients. For the pooled data on either sodium intake, a borderline significant correlation was present (R^2=0.217, P=0.095 and R^2=216, P=0.096 on LS and HS, respectively). However, the correlation disappeared after adjustment for population. The individual change in VEGF-C elicited by HS intake was not correlated with the change in MAP in either study population, separately or pooled (Figure 3). Furthermore, no significant associations were found between change in VEGF-C levels / total amount of VEGF-C and change in ECV, NT-proBNP, or body weight. VEGF-C levels, however, were significantly higher in CKD patients than in healthy subjects on either sodium intake (P=0.027 and P=0.006 on LS and HS, respectively).

DISCUSSION

We found that VEGF-C levels are modulated by salt intake in two different independent studies, with higher VEGF-C levels during high salt intake. First, in proteinuric CKD patients after two 6-week periods of dietary intervention, and second, in healthy subjects, after two 1-week periods of dietary intervention, albeit the latter of borderline statistical significance. In the CKD patients higher salt intake was associated with higher blood pressure, whereas in the healthy subjects the measured blood pressure was not affected by dietary salt, despite a rise in ECV.

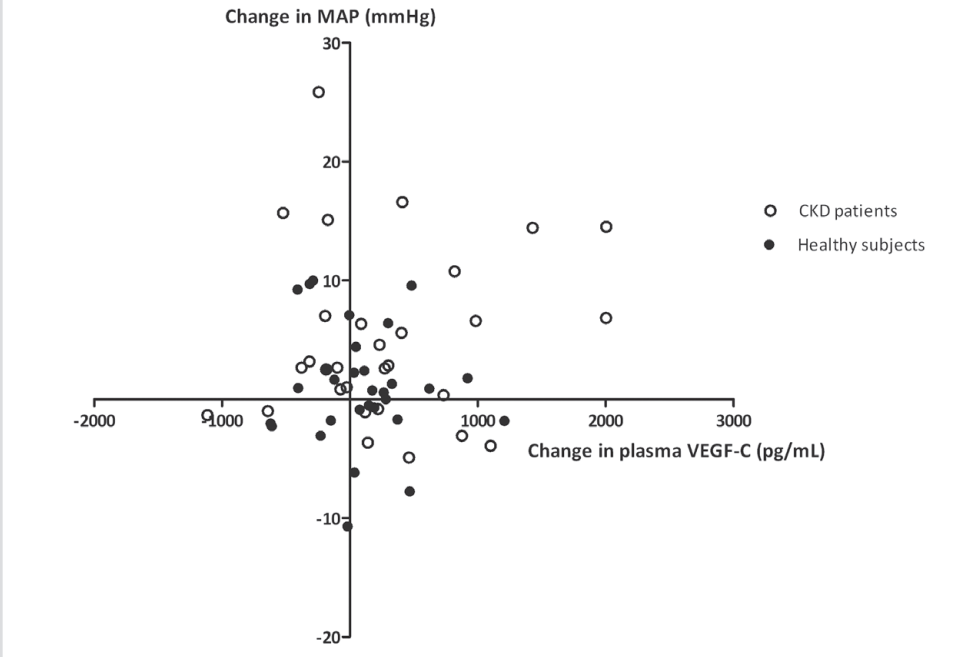
Animal studies have found that during high salt diet the content and polyanionic character of glycosaminoglycans increase, accompanied by hypertonic salt storage in the ensuing reservoir

Figure 2. Association between VEGF-C levels and MAP in CKD patients (N=32) and healthy subjects (N=31) during low during low and high sodium diet.



Abbreviations: LS: low sodium diet, HS: high sodium diet, VEGF-C: vascular endothelial growth factor C.

Figure 3. Association between change in VEGF-C levels and change in MAP in CKD patients (N=32) and healthy subjects (N=31).



Abbreviations: MAP: mean arterial pressure, VEGF-C: vascular endothelial growth factor C.

tissue.[7,16] VEGF-C, which is secreted by mononuclear phagocyte system (MPS) cells in response to interstitial hypertonicity, induces eNOS expression by binding to VEGFR-2[12] and stimulates lymphangiogenesis by binding to VEGFR-3.[13] The resulting vasodilatory response and electrolyte removal from the interstitium prevents a salt-sensitive blood pressure state.[4,5,17-19] This non-osmotic VEGF-C-macrophage-lymphangiogenesis pathway may act alongside the osmotic storage of salt that translates into ECV excess. As currently no methods are established for investigation of salt storage in patient-oriented research, we can only speculate that dietary salt induces salt storage in specific reservoirs as well. However, the close association between changes in dietary salt intake followed by parallel changes in plasma VEGF-C levels supports the notion that changes in MPS-derived VEGF-C levels might serve as a clinical indicator for salt overload and salt storage in humans. We believe that this new research area warrants further investigation in patient-oriented research.

In our proteinuric CKD patients blood pressure increased during the high salt diet, in line with the well-established salt-sensitivity of blood pressure in CKD,[20,21] and along with a rise in body weight and NT-proBNP, suggesting ECV expansion. Concomitantly, VEGF-C levels were increased, suggesting that high salt intake induces an extrarenal homeostatic pathway in these patients as well. This increase in VEGF-C was present despite the fact that during LS dietary sodium intake was substantially higher than the target of 50 mmol/d, thus limiting the difference with the HS period.

Animal data support a role for the VEGF-C-macrophage-lymphangiogenesis pathway in the protection against developing hypertension in response to a high sodium intake.[5] Furthermore, subjects with refractory hypertension show higher plasma VEGF-C levels than controls, suggesting that this pathway is relevant in the pathogenesis of human hypertension as well.[4] In our study populations we did not find a between-individual correlation between levels of VEGF-C and blood pressure, or between the responses of VEGF-C and blood pressure to high sodium when analyzing for individual responses, neither in the separate populations, nor for pooled data. This could implicate either absence of an association, or complete protection against a sodium-induced rise in blood pressure by the adaptive response of the VEGF-C-macrophage-lymphangiogenesis pathway. Whereas we want to emphasize that a head-to-head comparison between the two populations should be interpreted with caution, due to differences in the experimental design and patient characteristics, nevertheless it is noteworthy that VEGF-C levels were higher in the CKD patients, i.e. in the population where blood pressure was sodium sensitive.

The mechanism for the higher VEGF-C levels in CKD patients is of interest, but cannot be derived with certainty from our data. The data are consistent with the assumption that in CKD patients VEGF-C is stimulated more than in healthy controls on a similar sodium intake, which can be hypothesized to reflect a less effective response to sodium intake and hence a persisting stimulus. Whether this is due to differences in osmotic storage, non-osmotic storage, or to blunted sodium excretion in CKD leading to difference in overall sodium balance cannot be ascertained from our data. However, the higher NT-proBNP levels in CKD on each sodium intake are consistent with

a higher ECV and hence differences in overall balance and osmotically stored sodium in CKD patients.

The rise in blood pressure during high sodium in CKD patients suggests that the presumed extrarenal, MPS-driven regulatory mechanism is not sufficient to preclude a rise in blood pressure in response to high sodium. Of note, as VEGF-C reduces the permeability of the glomerular filtration barrier and promotes podocyte survival,[22,23] an increase in VEGF-C is theoretically expected to reduce proteinuria, independently of blood pressure. At variance with this consideration, in our patients proteinuria increased during high salt, probably secondary to the rise in blood pressure.

In an independent study in healthy subjects VEGF-C levels were also increased by a 1-week period on high salt diet, with a concomitant rise in the extracellular volume and creatinine clearance, whereas blood pressure was salt-resistant. These data suggest that the MPS-driven VEGF-C-macrophage-lymphangiogenesis regulatory pathway, which is specific for local tissue salt storage, is stimulated by high salt intake, alongside the conventional renal osmotic pathways of salt homeostasis. The rise in creatinine clearance can be considered part of the integrative homeostatic response to high sodium, and is considered instrumental in facilitating excretion of the excess sodium, and sodium resistance of blood pressure. This is consistent with our current observation of a rise in creatinine clearance in our, sodium resistant, healthy subjects, and a smaller, non-significant rise in creatinine clearance in our, sodium-sensitive, CKD patients. Of note, we previously demonstrated that the rise in GFR on high sodium closely corresponds to the rise in extracellular volume, i.e. the osmotic storage pathway, in healthy subjects.[25]

Our data are the first to document an effect of salt intake on VEGF-C, a crucial step in the newly identified VEGF-c-macrophage-lymphangiogenesis pathway as an extrarenal homeostatic mechanism in the response to an increase in salt intake in humans, in a salt-sensitive as well as a salt-resistant condition. Unfortunately, we have no data on total body composition and salt content. Furthermore, it would be of great interest to directly monitor local interstitial changes in humans during dietary salt intervention in future research.

To conclude, VEGF-C levels are increased by high salt diet in proteinuric CKD patients and in healthy subjects, supporting a role for VEGF-C mediated interstitial regulatory mechanisms in salt homeostasis in humans. Considering the rise in blood pressure during high salt diet, this buffering mechanism for salt-sensitive hypertension appears to be insufficient in proteinuric CKD patients. Future studies should investigate the clinical relevance, the reasons for failure in CKD, and potential targets for intervention, of VEGF-C mediated interstitial electrolyte- and volume homeostasis in humans.

Disclosures

None to declare.

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REFERENCES

1. Adrogue HJ, Madias NE. Sodium and potassium in the pathogenesis of hypertension. *N Engl J Med* 2007; 356:1966-1978.
2. Guyton AC, Coleman TG, Cowley AW, Jr., Liard JF, Norman RA Jr., Manning RD Jr. Systems analysis of arterial pressure regulation and hypertension. *Ann Biomed Eng* 1972; 1: 254-281.
3. Visser FW, Boonstra AH, Lely TA, Boomsma F, Navis G. Renal response to angiotensin II is blunted in sodium-sensitive normotensive men. *Am J Hypertens* 2008; 21: 323-328.
4. Machnik A, Neuhofer W, Jantsch J et al. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med* 2009; 15: 545-552.
5. Machnik A, Dahlmann A, Kopp C et al. Mononuclear phagocyte system depletion blocks interstitial tonicity-responsive enhancer binding protein/vascular endothelial growth factor C expression and induces salt-sensitive hypertension in rats. *Hypertension* 2010; 55: 755-761.
6. Go WY, Liu X, Roti MA, Liu F, Ho SN. NFAT5/TonEBP mutant mice define osmotic stress as a critical feature of the lymphoid microenvironment. *Proc Natl Acad Sci U S A* 2004; 101: 10673-10678.
7. Schafflhuber M, Volpi N, Dahlmann A et al. Mobilization of osmotically inactive Na+ by growth and by dietary salt restriction in rats. *Am J Physiol Renal Physiol* 2007; 292: F1490-F1500.
8. Kerjaschki D. The crucial role of macrophages in lymphangiogenesis. *J Clin Invest* 2005; 115: 2316-2319.
9. Schoppmann SF, Birner P, Stockl J et al. Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *Am J Pathol* 2002; 161: 947-956.
10. Heer M, Baisch F, Kropp J, Gerzer R, Drummer C. High dietary sodium chloride consumption may not induce body fluid retention in humans. *Am J Physiol Renal Physiol* 2000; 278: F585-F595.
11. Titze J, Maillet A, Lang R et al. Long-term sodium balance in humans in a terrestrial space station simulation study. *Am J Kidney Dis* 2002; 40: 508-516.
12. Lahdenranta J, Hagendoorn J, Padera TP et al. Endothelial nitric oxide synthase mediates lymphangiogenesis and lymphatic metastasis. *Cancer Res* 2009; 69: 2801-2808.
13. Joukov V, Kaipainen A, Jeltsch M et al. Vascular endothelial growth factors VEGF-B and VEGF-C. *J Cell Physiol* 1997; 173: 211-215.
14. Pimenta E, Gaddam KK, Oparil S et al. Effects of dietary sodium reduction on blood pressure in subjects with resistant hypertension: results from a randomized trial. *Hypertension* 2009; 54: 475-481.
15. Strazzullo P, Galletti F, Barba G. Altered renal handling of sodium in human hypertension: short review of the evidence. *Hypertension* 2003; 41: 1000-1005.
16. Titze J, Shakibaei M, Schafflhuber M et al. Glycosaminoglycan polymerization may enable osmotically inactive Na+ storage in the skin. *Am J Physiol Heart Circ Physiol* 2004; 287: H203-H208.
17. Tammela T, Alitalo K: Lymphangiogenesis. Molecular mechanisms and future promise. *Cell* 2010; 140: 460-476.
18. Leonard AM, Chafe LL, Montani JP et al. Increased salt-sensitivity in endothelial nitric oxide synthase-knockout mice. *Am J Hypertens* 2006; 19:1264-1269.
19. Tolins JP, Shultz PJ. Endogenous nitric oxide synthesis determines sensitivity to the pressor effect of salt. *Kidney Int* 1994; 46: 230-236.
20. De Nicola L, Minutolo R, Bellizzi V et al. Achievement of target blood pressure levels in chronic kidney disease: a salty question? *Am J Kidney Dis* 2004; 43: 782-795.
21. Ritz E, Dikow R, Morath C, Morath C, Schwenger V. Salt--a potential 'uremic toxin'? *Blood Purif* 2006; 24: 63-66.
22. Foster RR, Slater SC, Seckley J et al. Vascular endothelial growth factor-C, a potential paracrine regulator of glomerular

permeability, increases glomerular endothelial cell monolayer integrity and intracellular calcium. *Am J Pathol* 2008; 173: 938-948.
23. Foster RR, Satchell SC, Seckley J et al. VEGF-C promotes survival in podocytes. *Am J Physiol Renal Physiol* 2006; 291: F196-F207.
24. Vogt L, Waanders F, Boomsma F, de Zeeuw D, Navis G. Effects of dietary sodium and hydrochlorothiazide on the antiproteinuric efficacy of losartan. *J Am Soc Nephrol* 2008; 19: 999-1007.
25. Visser FW, Muntinga JH, Dierckx RA, Navis G. Feasibility and impact of the measurement of extracellular fluid volume simultaneous with GFR by 125I-iothalamate. *Clin J Am Soc Nephrol* 2008; 3:1308-1315.